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1) Am J Clin Pathol 1967 Mar;47(3):271-81
Glucose-6-phosphate dehydrogenase, the pentose phosphate cycle, and its place in carbohydrate metabolism.
Horecker BL

2) Folia Microbiol (Praha) 1982;27(6):365-9
Coordinate regulation of the pentose phosphate pathway and of the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (decarboxylating).
Pascual C, Herrera LS

Thank you,
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Folia Microbiol. 27, 365–369 (1982)

Coordinate Regulation of the Pentose Phosphate Pathway and of the Activity of Glucose-6-phosphate Dehydrogenase and 6-Phosphogluconate Dehydrogenase (Decarboxylating)

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ABSTRACT. The activities of glucose-6-phosphate dehydrogenase (GPD) and 6-phosphogluconate dehydrogenase (decarboxylating) (PGD) in *Saccharomyces cerevisiae* were found to change by a factor similar to the reported changes in the rate of the pentose phosphate pathway between cells grown in minimal medium and in rich medium. It is suggested that the rate of this pathway is regulated not only by the activity of GPD, a well-known key enzyme, but also by PGD. These two enzymes seem to function in a coordinated fashion.

In many biological systems the pentose phosphate pathway plays a key role in the formation of NADPH, pentoses and erythrose necessary for biosynthetic reactions (Pontremoli and Grazi 1969). Due to its properties and position in metabolism, a crucial function in the regulation of the pentose phosphate pathway has been attributed to glucose-6-phosphate dehydrogenase (GPD) (EC 1.1.1.49) (Afolayan 1972; Eggleston and Krebs 1974) while a secondary role has been ascribed to its partner enzyme, 6-phosphogluconate dehydrogenase (decarboxylating) (PGD) (EC 1.1.1.44). Both the induction of enzymes and the modulation of enzyme activity at the substrate level are considered to be important in regulating the capacity of these dehydrogenases (Sapag-Hagar *et al.* 1973; Holten *et al.* 1976).

In *Saccharomyces cerevisiae*, the composition of the medium used for cell growth constitutes an important factor involved in determining the rate of the pentose phosphate pathway. Gancedo and Lagunas (1973) demonstrated that the contribution of the pathway to glucose catabolism is greater in yeast grown in minimal medium than in yeast grown in an enriched medium. Since GPD and PGD have been assigned an important function in regulation it can be expected that the variation in the rate of the pathway observed by Gancedo and Lagunas is due to changes in the activities of the dehydrogenases. Evidence of this prediction as well as a study of several factors involved in the regulation of PGD is reported here.

MATERIALS AND METHODS

Strain. Saccharomyces cerevisiae 196-2 (α *his6*) was obtained from M. Luzzati.

Media. The complete medium (YP) contained (in g/L): yeast extract 10, peptone 10, glucose 20 or 100. The minimal medium was prepared as described by Galzy and

TABLE I. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (decarboxylating) in cells grown in different media

Medium	Substrate	nkat/mg	
		GPD	PGD
Complete	2 % glucose	1.87	2.25
	10 % glucose	2.12	2.57
	10 % glucose[a]	2.93	2.58
	3 % ethanol	4.18	3.02
Minimal	2 % glucose	4.90	4.67

[a]Cells harvested during stationary phase.

Slonimski (1957) supplemented with histidine (10 mg/mL). Depending on the type of experiment, other amino acids, tryptone, and purine and pyrimidine bases were supplied to the minimal medium.

Yeast growth. The strains were incubated at 30 °C with shaking at approximately 1.5 Hz and absorbance was read in a Spectronic-100 (Bausch and Lomb) spectrocolorimeter. They were harvested at mid-exponential phase.

Enzyme preparation and assay. Ten cells per nL were inoculated in 200 mL of the medium specified above and grown in conical flasks at 30 °C for 18 to 24 h with shaking. The cells were harvested by centrifugation, washed twice with deionized water and resuspended in 5 mM glycylglycine buffer (pH 7.6).

Cells were disrupted in a Braun cell homogenizer model MSK (Browill) for 80 s (50 s first velocity, 30 s second velocity). During disruption the flask was cooled by a stream of CO_2. The cell extract was immediately centrifuged (25 000 *g*, 20 min) in a MSE HS-18 centrifuge. The supernatant was desalted in a PD-10 Sephadex G-25 column previously equilibrated with buffer. Glucose-6-phosphate dehydrogenase activity was determined according to Kuby and Noltmann (1966) and 6-phospho-

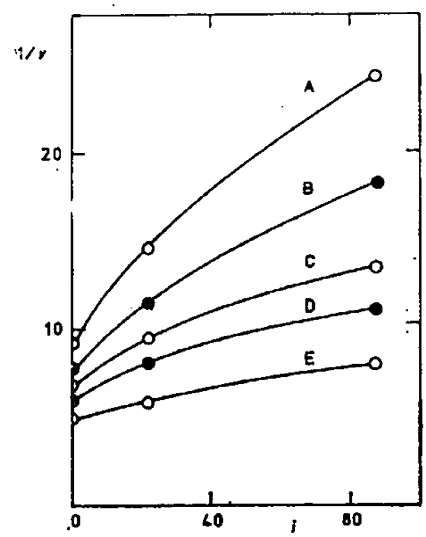

FIG. 1. Dixon plot (initial rate v expressed in ΔA_{340} per min) against concentration of NADPH (i) in μM. Curves A–E were obtained at the following concentrations of NADP as substrate (μM): 16.8, 33.5, 41.9, 67.1, and 167.7.

TABLE II. Effect of nitrogenous compounds on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (decarboxylating) activities in a medium with 2 % glucose

Medium	Addition	nkat/mg	
		GPD	PGD
Minimal	none	5.55	5.40
	tryptone and tryptophan	3.12	2.50
	purines and pyrimidines	4.85	5.22
	tryptone, tryptophan, purines and pyrimidines	3.78	2.77
Complete	none	2.60	2.60

gluconate dehydrogenase was assayed according to Pontremoli and Grazi (1966) by following the rate of reduced NADPH production in a Unicam SP 1800 spectrophotometer with recorder at 340 nm. Protein was determined by the biuret method (Layne 1957) using bovine serum albumin as standard.

Reagents. Yeast extract and peptone were from Oxoid, D-glucose and amino acids and bases were from British Drug Houses; 6-phosphogluconate, glucose 6-phosphate (disodium salt) and $MgCl_2$ were from Koch-Light; NADP and glycylglycine were from Merck.

RESULTS

When yeast cells are grown in minimal medium containing glucose, the specific activities of GPD and PGD are 2.6 and 2.1 times higher, respectively, in relation to those in a rich medium (Table I). This agrees well with the values reported by Lagunas and Gancedo (1973) where the contribution of the oxidative hexose monophosphate pathway to glucose catabolism is 2.8 times higher in yeast grown in minimal medium than in rich medium.

TABLE III. Effect of amino acids on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (decarboxylating) from cells grown in minimal medium with 2 % glucose

Amino acid added	nkat/mg	
	GPD	PGD
Tryptone plus Trp	1.87	2.28
Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val	2.22	2.45
Glu, His, Ile, Lys, Phe, Pro, Thr, Val	3.30	3.32
Ala, Arg, Asp, Cys, Gly, His, Leu, Met, Trp, Tyr, Ser	2.70	2.80
His, Phe, Trp, Tyr	5.22	4.23
His	4.93	4.10

TABLE IV. Effect of various compounds on 6-phosphogluconate dehydrogenase (decarboxylating) activity

Compound added	Concentration mM	PGD activity nkat/mg
None	—	2.55
Fructose 1,6-bisphosphate	0.34	1.53
GTP	0.31	1.78
NaH_2PO_4	0.21	1.98

Since the main components present in rich medium but absent from minimal medium are amino acids and purine and pyrimidine bases one might expect any one of these compounds to change the enzyme activities. Table II suggests that the amino acids in the medium included in the form of tryptone and tryptophan are responsible for these changes. Further confirmation is obtained from the results of Table III, since the presence in minimal medium of a mixture of 18 amino acids caused a similar effect on enzyme activities as growth in rich medium. However, when fewer amino acids were added to the minimal medium, variable values of enzyme activities were obtained.

Modulation of GPD and PGD activities by several physiological effectors and especially by the $NADP^+/NADPH$ ratio has been shown to be of primary importance in the regulation of the pentose phosphate dehydrogenases from different sources (Pontremoli and Grazi 1969; Tanaka *et al.* 1976; Dyson and D'Orazio 1971; Pearse and Rosemeyer 1974). These considerations led us to investigate whether any of these factors had an influence at the level of PDG activity in *S. cerevisiae*. We found NADPH to be a potent inhibitor of the enzyme, competing with $NADP^+$ in a partial fashion (*see* the nonlinear Dixon plot in Fig. 1). While the K_m for NADP was 26 μM, the K_i for NADPH read from a Lineweaver-Burk plot was 14 μM. This value is to be compared with that of human erythrocyte PGD where a K_i of 30 μM for NADPH was reported by Pearse and Rosemeyer (1974). However, considering the partial character of the inhibition we derived (*see* Kotyk and Horák 1977) the true K_i to be only 11.5 μM.

Table IV shows that phosphate ions, GTP and fructose 1,6-bisphosphate also inhibited PGD activity.

DISCUSSION

There is good agreement that the pentose phosphate pathway provides most if not all of the NADPH that is needed by the cells of *S. cerevisiae* when grown on glucose (Lagunas and Gancedo 1973). NADPH, ribose 5-phosphate and erythrose 4-phosphate, known intermediates of the pentose phosphate pathway, are needed for the biosynthesis of several amino acids (Mahler and Cordes 1971). Therefore, for yeast growing in a medium supplemented with amino acids, a great decrease of cellular NADPH requirement can be expected and actually the activities of GPD and PGD decrease. The activities of these enzymes change by a factor similar to the one reported for the change in the rate of the pathway between cells grown in minimal medium and rich medium. The interrelations in the conversion of one amino acid to another as well as the complexity in the regulation of its metabolism which is not completely elucidated makes it difficult to attribute a single amino acid or even a group of amino acids which utilizes NADPH for its biosynthesis, the ability to change the activities of GPD and PGD.

The finding of the inhibition of PGD by NADPH, fructose 1,6-bisphosphate, GTP and phosphate could be of significance in the regulation of enzyme activity by a fine control mechanism whereby instant changes of PGD activity could take place. Particularly interesting is the inhibition caused by fructose 1,6-bisphosphate since this compound exerts a positive effect on two principal glycolytic enzymes of *Saccharomyces cerevisiae:* 6-phosphofructokinase (EC 2.7.1.11) (Sols and Salas 1969) and pyruvate kinase (Gancedo *et al.* 1967). Therefore, a change in the concentration of this metabolite could regulate the level of glucose 6-phosphate which enters the glycolytic and pentose phosphate pathways. A similar view was put forth by Pearse and Rosemeyer (1974) for human erythrocytes. The above evidence supports the idea that not only GPD but also PGD can play a role in regulating the rate of the pentose phosphate pathway in yeast.

The authors wish to express their thanks to Prof. A. Kotyk for his useful advice and critical comments concerning this work.

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Thank you,
David Steadman